From Dianna 12/1/99 ** my copy *

TERATOLOGY 50:19–26 (1994)

Prevention of Fluvastatin-Induced Toxicity, Mortality, and Cardiac Myopathy in Pregnant Rats by Mevalonic Acid Supplementation

ROMAN V. HRAB, HOWARD A. HARTMAN, AND RAYMOND H. COX, JR.
Regulatory Toxicology, Drug Safety Department, Sandoz Research Institute, Sandoz Pharmaceuticals Corporation, East Hanover, New Jersey 07936

ABSTRACT Mevalonic acid is a product of the enzyme HMG-CoA reductase which is essential for cholesterol biosynthesis. Fluvastatin (Sandoz compound XU 62-320) is a potent inhibitor of this enzyme and, hence, mevalonic acid production. In three separate studies, oral administration of fluvastatin at 12 and 24 mg/kg/day to mated rats from day 15 of gestation through weaning resulted in unanticipated maternal mortality at the time of parturition and during lactation. Microscopic evaluations performed in two studies revealed significant cardiac myopathy in the dying animals. Drug-related clinical signs, significant maternal body weight loss, and an increase in stillborn pups and neonatal mortality were also noted at one or both dose levels. Supplementation of fluvastatin administration with 500 mg/kg b.i.d. of mevalonic acid completely blocked and/or ameliorated the mortality, cardiac myopathy, and other adverse effects. These studies indicate that the adverse maternal effects observed with fluvastatin before or following parturition resulted from exaggerated pharmacologic activity at the dose levels administered, i.e., inhibition of the enzyme HMG-CoA reductase, its immediate product mevalonic acid, and cholesterol biosynthesis.

? cardiac myopathy

Fluvastatin (Sandoz compound XU 62-320) is a synthetic potent inhibitor of hydroxymethylglutaryl coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme in cholesterol biosynthesis (Engstrom et al., '88; Kathawala et al., '88: Kathawala, '91). Administration of fluvastatin in rats, dogs, and monkeys induces significant reductions in serum total cholesterol, low-density lipoprotein cholesterol, and serum triglyceride levels (Engstrom et al., '88). During the safety assessment of fluvastatin, nonclinical studies were performed to evaluate the effect of this compound on fertility, reproductive performance, and teratogenicity in rats and rabbits. There were no adverse effects on fertility or reproductive performance up to the highest dose levels tested in male (20 mg/kg/day) and female (6 mg/kg/day) rats, and no evidence of teratogenic activity in rats at doses up to 36 mg/kg/day or rabbits at doses up to 10 mg/kg/day (R.V. Hrab, unpublished data). However, in a perinatal/postnatal study (Segment III), 12 and 24 mg/kg/day of fluvastatin administered to pregnant rats from day 15 pc (post coitus) through weaning resulted in maternal mortality at or near term and during the postpartum period. This mortality was replicated in a subsequent follow-up study, and microscopic tissue evaluation revealed the occurrence of cardiomyopathy only in the dying animals. No cardiac pathology was detected in nonpregnant animals in these studies with fluvastatin. As in previous rat studies (Stoll et al., '88), forestomach epithelial hyperplasia and hyperkeratosis were found. These have been shown to be specific to rodents and to be a result of contact irritation by fluvastatin (Robison et al., '94).

Coadministration of mevalonic acid, the immediate product of the enzyme HMG-CoA reductase, has been shown to prevent or antagonize various organ toxicities induced in rats and rabbits with other HMG-CoA reductase inhibitors (MacDonald et al., '88; Kornbrust et al., '89). In pregnant rats, it has been shown that 500 mg/kg b.i.d. of mevalonic acid suppressed the teratogenicity of mevinolinic acid, an inhibitor of HMG-CoA reductase, when administered prior to and approximately 5 hr after administration of mevinolinic acid (Minsker et al., '83). Since fluvastatin is an inhibitor of the enzyme HMG-CoA reductase and therefore of mevalonic acid production, a relationship was considered to exist between the exaggerated pharmacologic activity associated with HMG-CoA reductase inhibition by fluvastatin and the occurrence of maternal mortality and/or cardiac lesions. The study reported herein was designed to determine whether coadministration of me-

Received October 14, 1993; accepted February 25, 1994.

Address reprint requests to Roman V. Hrab, Regulatory Toxicology, Drug Safety Department, Sandoz Research Institute, Sandoz Pharmaceuticals Corporation, East Hanover, NJ 07936.

This paper was submitted for publication shortly after the untimely death of Dr. Cox in August 1993.

TABLE 1. Daily dosing schedule

Group	First dose administration	Second dose administration (approx. 1/2 hr. after first dose)	Third dose administration (approx. 5 hr. after second dose)
I	Deionized H_2O	1% CMC	Deionized H_2O
II	Mevalonic acid 500 mg/kg	1% CMC	Mevalonic acid 500 mg/kg
III	Mevalonic acid 500 mg/kg	Fluvastatin 12 mg/kg/day	Mevalonic acid 500 mg/kg
IV	Mevalonic acid 500 mg/kg	Fluvastatin 24 mg/kg/day	Mevalonic acid 500 mg/kg
V	—	Fluvastatin 12 mg/kg/day	—
VI	—	Fluvastatin 24 mg/kg/day	—

valonic acid with fluvastatin from day 15 of gestation through day 21 postpartum could inhibit or block the adverse effects noted with fluvastatin.

MATERIALS AND METHODS

Animals

One hundred twenty female rats (Charles River CD®-Sprague-Dawley-derived) obtained from Charles River Breeding Laboratories, Inc., Kingston, New York, were approximately 14 wk old at the start of the study. A group of males of the same strain and age, and from the same supplier, was used for mating, during which each female was housed with one male. Observation of vaginal plugs the following morning was considered evidence of mating and day 0 of gestation or day 0 post coitus (pc). The day on which pups were born was designated day 0 postpartum (pp), while day 21 pp was the day of weaning. As the females were mated, they were randomly assigned to six groups until 20 rats per group were mated. After mating, females were housed individually in suspended stainless steel wire-bottom cages in a temperature and humidity controlled room with a 12-hr light and 12-hr dark cycle. On day 20 of gestation, the rats were transferred to transparent plastic cages with bedding consisting of hardwood chips. Certified Purina rodent chow (pellets) and water (provided via automatic watering device) were supplied ad libitum.

Compounds administered

Lot 28 of fluvastatin (XU 62-320) was used in this experiment. Purity was determined by thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC) to be 99+%. DL-mevalonic acid lactone was obtained from Fluka Chemical Corp. (Ronkonkoma, NY), and purity was found to be 96+% as determined by HPLC.

Dosage administration

Dosing solutions of fluvastatin were prepared fresh daily in 1% carboxymethylcellulose (CMC). Mevalonic acid was prepared fresh twice daily in deionized water. Individual animal doses were calculated and administered daily (to females only) via gavage from day 15 of gestation through day 21 postpartum at a volume of 10 ml/kg (Table 1). Dose calculations were based on the daily body weight from day 15 of gestation until delivery (or day 28 pc for those animals which did not deliver). Following delivery (day 0 pp) through weaning (day 21 pp), the dose calculations were based on the most recently recorded body weight. Control rats received three separate doses: 10 ml/kg b.i.d. of deionized water and 10 ml/kg/day of 1% CMC, also based on the same body weight schedules as in the dose groups. Concentrations of the dosing solutions were assayed and verified four times during the study.

Examinations

Individual clinical observations were recorded at least once daily. Body weights were recorded on days 0, 7, and daily from days 15 through 20 of pregnancy. After each animal delivered, maternal body weights were recorded on days 0, 7, 14, and 21 pp. Food consumption was recorded only during gestation on days 0, 7, 15, and 20 pc.

Following delivery of each litter, viability, clinical signs, and external examinations of pups were recorded on days 0 pp through 21 pp. The sex and individual weights of all viable pups were recorded on days 0, 1, 4, 7, 14, and 21 pp. Pups stillborn or found dead during the postpartum period and not autolyzed or cannibalized were examined, identified, and preserved in neutral buffered 10% formalin. Dams dying spontaneously or sacrificed moribund were necropsied. Animals which did not deliver were necropsied on day 28 pc. On day 21 pp, all remaining dams and pups were euthanized, necropsied, and examined. Pups with lesions as well as several control group pups were identified and saved in neutral buffered 10% formalin. All other pups were discarded.

Post mortem examinations

Following induction of deep surgical anesthesia using excess CO_2, animals were euthanized by severing the axillary vessels for exsanguination. Terminal body weight was recorded for each dam and a thorough dissection was performed on all surviving dams at weaning (day 21 pp). All gross lesions and approximately 40 representative tissue specimens were collected and preserved in neutral buffered 10% formalin.

Spontaneously dying animals were dissected and a complete set of tissue specimens, including the gravid reproductive tracts, were collected and preserved. Nonpregnant animals were sacrificed on day 28 pc. Since the treatment-related cardiac findings occurred only in

TABLE 2. Cause of death of spontaneously dying animals

Dose group	Total number of females that died on study	Pregnant females that died before delivery on day 22 pc	Pregnant females that died postpartum	Nonpregnant females dying during experiment
I Control	0	0	0	0
II Mevalonic acid 500 mg/kg	2	0	1 (sac. moribund day 1 pp)[4]	1 (day 26 pc)[5]
III Fluvastatin 12 mg/kg, mevalonic acid 500 mg/kg	1	0	0	1 (day 18 pc)[4]
IV Fluvastatin 24 mg/kg, mevalonic acid 500 mg/kg	2	0	0	1 (day 19 pc)[3] 1 (elective sac. day 15 pc)[7]
V Fluvastatin 12 mg/kg	3	1[1]	1 (day 4 pp)[1] 1 (day 13 pp)[6]	0
VI Fluvastatin 24 mg/kg	8	1[2]	1 (day 2 pp)[2] 1 (day 5 pp)[2] 1 (day 6 pp)[2] 2 (sac. moribund day 10 pp)[2] 1 (day 12 pp)[2] 1 (day 14 pp)[2]	0

[1]Cause could not be determined.
[2]Heart lesion (vacuolar degeneration, and/or myocarditis probably related to death).
[3]Intubation accident, lesions seen by gross or microscopic examination.
[4]Intubation accident based on clinical observations and gross or microscopic examination.
[5]Possible intubation accident, although microscopic findings were not conclusive.
[6]Intubation accident based on clinical observations, although microscopic findings were not conclusive.
[7]Broken, malaligned incisors; animal in poor health.

pregnant animals, data from the nonpregnant animals were considered separately.

Based on results from previous studies, microscopic evaluations were limited to the hearts and stomachs from all animals. For spontaneously dying or animals sacrificed moribund, the tissue evaluation included gross lesions, lungs, trachea, thymus with mediastinum, and liver.

Statistical analysis

All statistical evaluations of data compared treatment groups with controls at $P \leq 0.05$ and $P \leq 0.01$ levels of significance, with a two-tailed analysis. Maternal body weight, duration of gestation, number of pups delivered, live pups per litter, pup weight, and implantation sites were evaluated by analysis of variance (ANOVA) followed by Dunnett's Test. Fisher's Exact Test was used to evaluate maternal fertility and reproductive performance, numbers of dead pups, and sex ratio, as well as neonatal necropsy examination data. Maternal terminal body weights were evaluated by a one-way ANOVA followed by Duncan's Multiple Range Test.

RESULTS

Maternal mortality and clinical observations

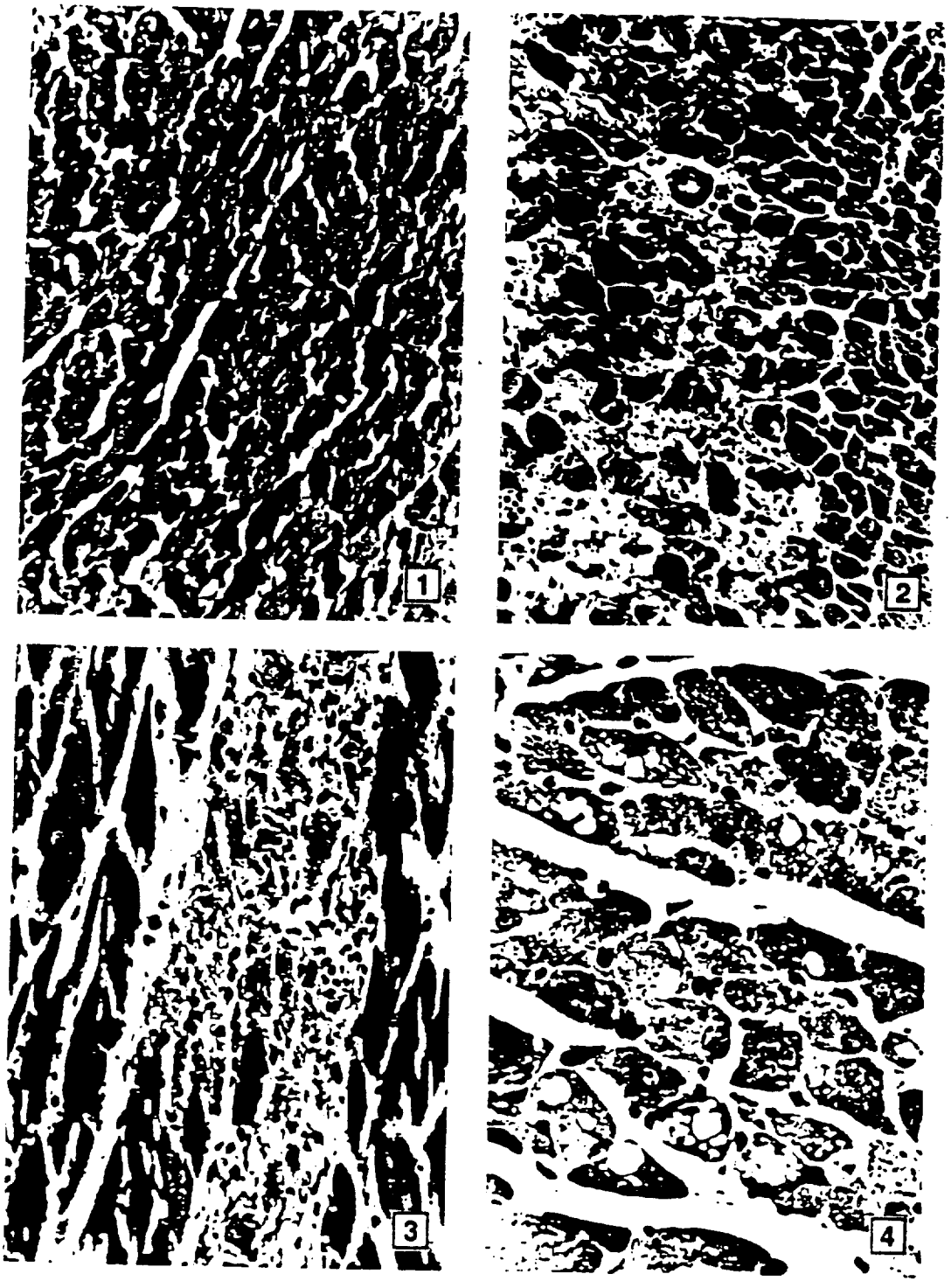
Drug-related maternal mortality occurred in animals receiving 12 and 24 mg/kg/day of fluvastatin where 2 out of 17 and 8 out of 17 pregnant animals, respectively, died or were sacrificed moribund (Table 2). One animal from each of these groups died on day 22 of gestation without completing delivery but had full-term fetuses in utero. Others died within the first 2 wk postpartum. The cause of death of the 8 animals administered fluvastatin at 24 mg/kg/day was related to the presence of cardiomyopathy characterized by hyaline, granular or vacuolar degeneration, and/or myocarditis (Fig. 2–5). The cause of death of the 2 rats administered fluvastatin at 12 mg/kg/day could not be determined.

Necropsy examination of the 2 animals that died on day 22 of gestation revealed dead, morphologically normal full-term fetuses in the uterus, indicating that they were probably alive up to the time of the dam's death. Furthermore, drug-related clinical observations were seen in the 24 mg/kg/day fluvastatin animal which included decreased locomotor activity (on days 20 and 21 pc), splayed (extended) hindlimbs, tremors, ataxia, labored breathing, salivation, lacrimation, and disorientation on day 21 pc. Similar signs were seen in the 12 mg/kg/day animal on day 22 pc prior to death.

Other animals from the 12 and/or 24 mg/kg/day fluvastatin groups also exhibited various drug-related clinical signs including ataxia, impairment/loss of righting reflex, decreased locomotor activity, hunched or flattened body position, splayed (extended) hind limbs, ptosis, tremors, disorientation, labored breathing, skin pallor, and dehydration.

Maternal body weight and food consumption

No treatment-related differences in maternal body weight gain were noted during gestation days 15–20, the initial period of administration of fluvastatin and/or mevalonic acid (Table 3). During the first 7 days after delivery, a transient, statistically significant ma-



Figs. 1–4.

Fig. 5. 24 mg/kg: death on day 14 pp. In some high-dose animals dying in the later postpartum period, myocardial lesions encountered were often more complex, frequently characterized by areas of myofiber disintegration accompanied by inflammatory infiltrates and interstitial edema. The affected areas ranged from limited to widespread. Not seen in earlier myocardial lesions was the occurrence of conspicuous cytoplasmic vacuolization illustrated in this figure (× 400).

ternal body weight *loss* of 5% and 9% was apparent in the 12 and 24 mg/kg/day fluvastatin dose groups, respectively, compared to a 4% *gain* in controls. A 2% body weight *loss* occurred in animals receiving the 24 mg/kg/day dose level of fluvastatin supplemented with mevalonic acid. During the next 7 days (days 7–14 pp), a body weight gain was evident in all groups. However, considering the first 2 wk postpartum (days 0–14), an overall body weight loss was nevertheless apparent in the 2 fluvastatin groups (Table 3).

Subsequently, during days 14–21 pp, a slight maternal body weight loss was observed in all groups including controls. Maternal weight loss is not uncommon during this time period and is probably due to a greater nutritional demand on the dams resulting from increased lactation, combined with more ingestion of feed by the pups and therefore less by the dams. Although not statistically significant, a slight decrease in food consumption of approximately 11% was evident during the treatment period from days 15–20 of gestation in both groups administered 24 mg/kg/day of fluvastatin, with and without mevalonic acid (Table 3).

Fertility and reproductive performance

No remarkable variation in pregnancy rate occurred among the groups. Considering the mean length of the gestation period of those animals completing delivery, there was no treatment-related variation among groups (Table 4). A slightly lower gestation index in Group VI (fluvastain at 24 mg/kg/day) reflects the one animal which had no viable pups, 5 stillborn pups, and 7 dead pups of unknown viability status at birth.

Postnatal litter data

The mean number of liveborn pups was lower in both groups administered fluvastatin alone (Groups V and VI) when compared to concurrent controls (Table 5). An increased incidence of stillborn pups occurred in the 12 and 24 mg/kg/day fluvastatin Group V (7%) and Group VI (22%), compared to a range of 2–3% in controls and Groups II, III, and IV. There was no evidence to suggest any remarkable increase in in utero postimplantation embryo/fetal mortality (i.e., early or late resorptions) in any of the groups, either before or after initiation of fluvastatin and/or mevalonic acid administration (Table 5). Neonatal mortality of 15% and 56% was observed through weaning in both 12 and 24 mg/kg/day fluvastatin Groups V and VI, respectively (Table 5), with a statistically significant increase noted at 8–14 days postpartum in Group V and as early as 1–4 days postpartum in Group VI. Although the overall neonatal mortality in Group IV (24 mg/kg/day fluvastatin supplemented with mevalonic acid) was also statistically significant, it was minimal.

Drug-related lower birth weights as well as lower neonatal body weights during the lactation period were evident in both 24 mg/kg/day fluvastatin dose groups, with and without mevalonic acid supplementation.

The most prominent neonatal clinical observations occurred in Group VI (24 mg/kg/day of fluvastatin) where several litters, whose dams were adversely af-

Fig. 1. Control animal: typical appearance of nontreated control myocardial tissue (× 200).

Fig. 2. 24 mg/kg: death on day 21 pc before delivery. An example illustrating the significant but focal nature of myocardial hyaline degeneration, interstitial edema, and evidence of actual myofiber loss encountered in dying high-dose animals. Lesions of this type (left half of illustration) were detected only in animals dying during delivery or immediately postpartum. The myocardium in the right half of this illustration is unaffected (× 200).

Fig. 3. 24 mg/kg: death on day 22 pc before delivery. Discrete area of significant myocardial necrosis with mononuclear phagocytic infiltration. Affected myofibers have undergone granular degeneration. Adjacent myofibers are normal. Multiple foci of this type were seen elsewhere in the myocardium of this animal (× 200).

Fig. 4. 24 mg/kg: sacrificed moribund on day 10 pp. Conspicuous myofiber cytoplasmic vacuolization was frequently noted in animals dying later in the postpartum period. These cytoplasmic alterations were unaccompanied by inflammation or other evidence of myofiber degeneration. The distribution of affected fibers ranged from limited areas to widespread regions of myocardium. The vacuoles themselves were found to be devoid of fat or glycogen (× 400).

TABLE 3. Percent body weight change and relative food consumption compared to controls

Group	I Control	II Mevalonic Acid	III Fluvastatin + Mevalonic Acid	IV Fluvastatin + Mevalonic Acid	V Fluvastatin	VI Fluvastatin
	0 mg/kg/day	500 mg/kg b.i.d.	12 mg/kg/day + 500 mg/kg b.i.d.	24 mg/kg/day + 500 mg/kg b.i.d.	12 mg/kg/day	24 mg/kg/day
% body weight change:						
days 15–20 pc	18	18	18	16	16	16
0–7 pp	4	3	2	−2	−5*	−9*
0–14 pp	8	4	4	2	−1**	−3*
7–14 pp	4	1	2	4	4	1
14–21 pp	−5	−3	−4	−3	−3	−1
Relative food consumption:						
days 15–20 pc	100%	100%	96%	89%	93%	89%

*P ≤ 0.01.
**P ≤ 0.05.

TABLE 4. Fertility and reproductive performance

Group	I Control	II Mevalonic Acid	III Fluvastatin + Mevalonic Acid	IV Fluvastatin + Mevalonic Acid	V Fluvastatin	VI Fluvastatin
	0 mg/kg/day	500 mg/kg b.i.d.	12 mg/kg/day + 500 mg/kg b.i.d.	24 mg/kg/day + 500 mg/kg b.i.d.	12 mg/kg/day	24 mg/kg/day
No. of animals mated/group	20	20	20	20	20	20
# Pregnant	18	17	15	18	17	17
Pregnancy rate[1]	90%	85%	75%	90%	85%	85%
Gestation index[2]	100%	100%	100%	100%	100%	94%
No. of animals completing delivery	18	16[3]	15	18	16	16
Mean length gestation of animals completing delivery (days)	21.9	21.8	22.0	21.7	22.1	22.2
Range (days)	21–22	21–22	22	21–22	22–23	21–23

[1]Percent matings resulting in pregnancy; includes animals which died.
[2]Percent pregnancies resulting in litters with one or more viable offspring; does not include animals which died.
[3]Does not include one animal which delivered 4 stillborn, 4 dead, and 6 viable pups, but was sacrificed in a moribund condition on day 1 pp following a probable intubation accident on day 22 pc.

TABLE 5. Neonatal litter size and mortality

Group	Control	II	III	IV	V	VI
Mean no. of pups delivered	15.4	16.1	17.1	15.6	13.6	14.9
Mean no. of liveborn pups	15.1	15.9	16.7	15.2	12.6	11.1*
Mean no. of implantation sites	16.4	17.3	18.2	16.7	15.2	16.3
In utero post implantation loss	6.1%	6.9%	6.0%	6.6%	10.5%	8.6%
Overall neonatal mortality through weaning	3%	5%	6%	7%**	15%*	56%*

*P ≤ 0.01.
**P ≤ 0.05.

fected, had pups which appeared pale, thin, weak, and/or dehydrated. Necropsy examination of pups which were found dead during the lactation period, or were culled prior to weaning, showed that both groups administered fluvastatin alone (Group V and VI) had a higher incidence of pups without milk visible in the stomach.

Macroscopic and microscopic evaluation of spontaneously dying animals

No macroscopic evidence of treatment-related effects was noted other than the anticipated forestomach thickening present in 2 of 3 and 7 of 8 animals from the 12 and 24 mg/kg/day fluvastatin groups, respectively, which died or were sacrificed moribund.

TABLE 6. Time and incidence of treatment-related maternal mortality[1]

Study	Segment III			Segment III follow-up				Segment III mevalonate supplementation				
	Fluvastatin			Fluvastatin				MEV	Fluvastatin + MEV	Fluvastatin + MEV	Fluvastatin	
Dose (mg/kg)	2	12	24	2	6	12	24	500	12 + 500	24 + 500	12	24
Day 15–21 pc		2					2					
0 pp = 22 pc		2	4				4				1	1
23 pc		1	1									
1–4 pp			1				2				1	1
5–15 pp		1	6			1	3					6

[1]To facilitate simultaneous presentation of data, the control groups (all negative) were omitted.

Microscopic findings consisted of a variety of myocardial lesions in all dying Group VI (fluvastatin at 24 mg/kg/day) animals. The cardiomyopathy was characterized by focal myocarditis and lesions of myofiber hyaline, granular and vacuolar degeneration (Table 2). These findings were considered treatment-related and similar to those seen in a previous study in pregnant or lactating animals dying during or after parturition.

Two types of myocardial lesions were detected microscopically. In animals dying at and within a few days after parturition, the lesions were characterized by hyaline degeneration of isolated groups of myofibers accompanied by slight interstitial edema and hemorrhage (Fig. 2 and 3). The lesions typical of animals dying 3–4 days after parturition or later were characterized by widespread cytoplasmic vacuolar changes in the myofibers, i.e., the occurrence of large and small clearly defined round empty vacuoles (Fig. 4 and 5). Occasionally these were accompanied by a sarcolemmal response and inflammatory cell infiltrates, which suggested a myofiber proliferative or reparative response to the induction of the myocardial injury.

Cardiomyopathy was not present in the spontaneously dying animals which had been given a lower dose (12 mg/kg/day) of fluvastatin alone. All animals dying in the 24 mg/kg/day fluvastatin group had cardiac lesions. These lesions were considered related to the cause of death in these animals.

Terminal sacrifice animals

The only treatment-related effect noted occurred in all the groups which received fluvastatin. Most of the animals in Group III (14 out of 19), Group IV (17 out of 18), Group V (14 out of 17), and Group VI (11 out of 12) had varying degrees of forestomach thickening. No evidence of this effect was detected in Group II (mevalonic acid alone) or the controls. In Groups III and IV, supplementation with mevalonate did not influence the incidence of fluvastatin-induced forestomach thickening.

Microscopically, the forestomach thickening was characterized by epithelial hyperplasia/hyperkeratosis in all groups which received fluvastatin, including those receiving the mevalonic acid supplementation. No evidence of ulceration accompanied these hyperplastic lesions. The hyperplasia ranged from mild to moderate in degree. In the absence of specific quantitative measurements, there appeared to be correlation, with greater degrees of hyperplasia seen at the higher doses of fluvastatin in Groups IV and VI (24 mg/kg/day) compared to the response seen in Groups III and V (12 mg/kg/day). There did appear to be a lesser degree of hyperplasia/hyperkeratosis in the animals administered mevalonate and 12 mg/kg/day fluvastatin (Group III) compared to the low dose of fluvastatin alone (Group V).

Histological evidence of fluvastatin-induced myocardial fiber injury and/or inflammation was not present in the mevalonate-supplemented Groups III and IV. Likewise, in the surviving Group V and VI (fluvastatin at 12 and 24 mg/kg/day) animals, myocardial lesions were not detected.

DISCUSSION

The maternal toxic effects induced by fluvastatin reported herein had been encountered in two previous studies at similar dose levels, and consisted of maternal mortality at delivery and during the postpartum period (Table 6). Microscopic evidence of associated myocardial lesions was detected in the follow-up study. An increase in stillborn pups was observed with no evidence of earlier in utero fetal mortality, suggesting a toxic effect on the dams immediately prior to, or during, parturition. The absorption and disposition of fluvastatin have been studied in nonpregnant rats (Tse et al., '90) and in pregnant and lactating rats, and in suckling pups (A. Schweitzer, unpublished data). In all cases fluvastatin was rapidly eliminated with little or no accumulation in the tissues. Limited placental transfer resulted in very low levels in embryos or fetuses. Fluvastatin was detected in maternal milk and subsequently at measurable levels in suckling pups. However, the neonatal mortality observed soon after parturition, as well as during the lactation period, may be a reflection of adverse effects relative to deficient maternal lactation and lack of litter care as influenced by maternal toxicity.

The results indicate that fluvastatin-induced maternal and neonatal mortality could be completely blocked

and/or ameliorated by coadministration of mevalonic acid. Adverse effects on maternal body weight were nonexistent with mevalonic acid supplemented fluvastatin at 12 mg/kg/day and markedly improved at 24 mg/kg/day, although food consumption remained slightly decreased at 24 mg/kg/day despite supplementation with mevalonic acid. The decreased neonatal body weight gain during lactation at 24 mg/kg/day may be related to the reduced maternal food intake noted at this dose level.

While mevalonate supplementation prevented mortality and the development of heart lesions in Groups III and IV (fluvastatin at 12 and 24 mg/kg/day), it did not reduce the direct effect of fluvastatin on the gastric forestomach squamous epithelium, although the degree did appear somewhat lower in Group III. This is considered further evidence that hyperplasia/hyperkeratosis is the result of direct or contact irritation by fluvastatin on the squamous forestomach epithelium.

The fluvastatin-induced early cardiac lesions characterized by hyaline degeneration (before or at parturition) resembled those induced by isoproterenol (pregnant rats are uniquely sensitive)[1]. The postnatal lesions (vacuolar sarcoplasmic changes and myocarditis) appeared to be the result of sarcoplasmic fluid accumulation possibly related to alteration of cellular membrane structures.[1] The relationship or progression between pre- and postpartum lesions, if any, is not clear. Likewise, it is not known at what point in their development either would be reversible.

Several factors existed which probably contributed to the development of myocardial damage and/or death of the fluvastatin-treated animals. In addition to the concurrent stresses of pregnancy, parturition, and lactation, the exaggerated pharmacologic activity of fluvastatin, i.e., inhibition of mevalonate and cholesterol biosynthesis, was a significant factor. HMG-CoA reductase and its immediate product mevalonic acid play a key role in cholesterol biosynthesis and are essential for the synthesis of steroid hormones, cell membranes, cell growth, and normal cellular metabolism (Brown and Goldstein, '80; Alberts, '88; MacDonald et al., '88). It would not be unexpected, therefore, that inhibition of this critical enzyme would influence or disturb the dynamic events occurring in late gestation and at parturition. The data from Groups III and IV clearly indicate that mevalonate supplementation prevents mortality and the development of heart lesions. It is not clear from these studies exactly what the effect is in the myocardial tissues which allows development of the heart lesions, and why the restored or maintained levels of mevalonate suppress or block the development of these lesions.

[1]Wagner, B.M., Deputy Director, Nathan S. Kline Institute, NYU. Consultation at Sandoz, East Hanover, N.J. December 29, 1989.

LITERATURE CITED

Alberts, A.W. (1988) Discovery, biochemistry and biology of lovastatin. Am. J. Cardiol., *62*:10–15.

Brown, M.S., and J.L. Goldstein (1980) Multivalent feedback regulation of HMG CoA reductase, a control mechanism coordinating isoprenoid synthesis and cell growth. J. Lipid. Res., *21*:505–517.

Engstrom, R.G., D.B. Weinstein, F.G. Kathawala, T. Scallen, J.B. Eskesen, M.L. Rucker, R. Miserendino, and J. Babiak (1988) Hypolipoproteinemic activity of XU 62-320, a potent competitive inhibitor of HMG-CoA reductase. Eighth International Symposium on Atherosclerosis, Rome. October 9–13, 1988; 230 (abstract).

Kathawala, F.G., T. Scallen, R.G. Engstrom, D.B. Weinstein, H. Schuster, R. Stabler, J. Kratunis, J.R. Wareing, W.F. Jewell, L. Widler, and S. Wattanasin (1988) XU 62-320, an HMG-CoA reductase inhibitor, more potent than compactin and lovastatin. Eighth International Symposium on Atherosclerosis, Rome. October 9–13, 1988; 445 (abstract).

Kathawala, F.G. (1991) HMG-CoA reductase inhibitors: An exciting development in the treatment of hyperlipoproteinemia. Med. Res. Rev., *11*:121–146.

Kornbrust, D.J., J.S. MacDonald, C.P. Peter, D.M. Duchai, R.J. Stubbs, J.L. Germershausen, and A.W. Alberts (1989) Toxicity of the HMG-Coenzyme A reductase inhibitor, lovastatin, to rabbits. J. Pharmacol. Exp. Ther., *248*:498–505.

MacDonald, J.S., R.J. Gerson, D.J. Kornbrust, M.W. Kloss, S. Prahalada, P.H. Berry, A.W. Alberts, and D.L. Bokelman (1988) Preclinical evaluation of lovastatin. Am. J. Cardiol., *62*:16–27.

Minsker, D.H., J.S. MacDonald, R.T. Robertson, and D.L. Bokelman (1983) Mevalonate supplementation in pregnant rats suppresses the teratogenicity of mevinolinic acid, an inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A reductase. Teratology, *38*:449–456.

Robison, R.L., W. Suter, and R.H. Cox (1994) Carcinogenicity and mutagenicity studies with fluvastatin, a new entirely synthetic HMG-CoA reductase inhibitor. Fund. Appl. Toxicol. (manuscript accepted for publication).

Stoll, R.E., D.J. Ball, M. Evans, O. Hockwin, S. Roberts, R. Robison, L. Rubin, and C. Smith (1988) Subchronic target organ toxicity of XU 62-320 in the rat, mouse, dog and monkey. Eighth International Symposium on Atherosclerosis, Rome. October 9–13, 1988;902 (abstract).

Tse, F.L.S., H.T. Smith, F.H. Ballard, and J. Nicolletti (1990) Disposition of fluvastatin, an inhibitor of HMG-CoA reductase, in mouse, rat, dog, and monkey. Biopharm. Drug Dispos., *11*:519–531.